09/913955
JC05 Rec'd PCT/PTO 21 AUG 2001

We claim:

1. A soluble purified PHEX enzyme (secPHEX) and variants thereof, comprising a PHEX ectodomain or catalytic part thereof.

2. A soluble purified PHEX enzyme (secPHEX) and variants thereof, comprising a PHEX ectodomain or catalytic part thereof and a PHEX signal peptide/transmembrane region modified so as to confer solubility to said PHEX enzyme when expressed in a eukaryotic host, wherein said host is not a human being.

SUB A17

3. A mutant of the enzyme of claim 1 or 2, which is inactive but retains binding capacity to a ligand molecule to PHEX.

4. A mutant as defined in claim 3 consisting of PHEX enzyme having the glutamic acid residue at position 581 is mutated.

5. A mutant as defined in claim 3 consisting of PHEX enzyme having the glutamic acid residue at position 581 substituted with a hydrophobic amino acid residue.

6. A mutant as defined in claim 3 consisting of PHEX enzyme having the glutamic acid residue at position 581 substituted with a valine residue.

7. A nucleic acid which comprises a truncated PHEX gene sequence encoding PHEX membrane-anchor domain modified to include a cleavable signal peptide and PHEX C-terminal ectodomain is active or inactive.

8. A recombinant vector comprising the nucleic acid of claim 7.

9. A recombinant vector as defined in claim 8, which is an expression vector.

10. A eukaryotic host comprising the recombinant vector of claim 8, wherein said host is not a human being

11. A eukaryotic host comprising the recombinant vector of claim 9, wherein said host is not a human being.

12. A method for producing a soluble PHEX enzyme or an inactive mutant thereof, which comprises the steps of:

- allowing the eukaryotic host of claim 10 or 11 to express said nucleic acid, and
- recovering the soluble PHEX enzyme or mutant thereof as a secretion product of said host.

13. An antigenic composition, which comprises the enzyme of any one of claims 1 to 6.

14. An antibody capable of binding to PHEX and raised against the enzyme of any one of claims 1 to 6 or fragment thereof.

15. The antibody of claim 14, wherein said fragment extends from residue 121 to residue 294 of the amino acid sequence of PHEX.

16. An antibody as defined in claim 14, which is a monoclonal antibody.

17. An antibody as defined in claim 15, which is a monoclonal antibody.

18. The antibody of claim 16, which is a PHEX neutralizing antibody.

19. A hybridoma producing the antibody of any one of claims 16 to 18.

20. A composition comprising the enzyme of claim 1 or 2 or the nucleic acid of claim 7 and a pharmaceutically acceptable carrier.

SUB A3

21. A composition comprising the enzyme of any one of claims 3 to 6 and a pharmaceutically acceptable carrier.

22. A composition comprising the antibody of any one of claims 14 to 18 and a pharmaceutically acceptable carrier.

23. A diagnostic reagent for detecting the presence or amount of PHEX, comprising the antibody of any one of claims 14 to 18.

24. A diagnostic kit for detecting the presence or amount of PHEX comprising the antibody of any one of claims 14 to 18.

25. The diagnostic kit of claim 24, which further comprises a soluble PHEX enzyme.

SUB A4

26. A method for detecting the presence or an amount of PHEX in a sample, which comprises the steps of:
- contacting said sample with the antibody of any of any one of claims 14 to 18 in conditions such that the immune complex can form; and
- detecting the immune complexes as an indication of the presence or amount of PHEX in said sample.

SUB A4 CONT

27. A device for purifying PHEX or a mutant thereof which comprises the antibody of any one of claims 14 to 18.

28. A device for screening PHEX ligands, which comprises the soluble PHEX enzyme or a mutant thereof as defined in any one of claims 1 to 6.

29. The device of claim 27, wherein said antibody is fixed onto a solid support.

30. The device of claim 28, wherein said PHEX enzyme or mutant is fixed onto a solid support.

31. The device of claim 30, wherein said PHEX enzyme or mutant is fixed onto the solid support through its binding to an anti-PHEX antibody itself fixed onto said solid support.

32. The device of claim 30, wherein said PHEX enzyme or mutant is fixed onto the solid support through a C-terminal amino acid extension ending with a residue or group capable of coupling PHEX to the solid support.

SUB A5

33. A method for obtaining a PHEX ligand which comprising the steps of:

contacting a sample containing one or more molecules with a PHEX enzyme or mutant as defined in any one of claims 1 to 6 in conditions such that binding of said one or more molecules with PHEX can occur;

detecting said binding as an indication of the presence of a PHEX ligand in said sample; and

SUB A5 CONT.

selecting said PHEX ligand.

34. The method of claim 33, wherein said ligand is a PHEX inhibitor or substrate.

35. A method for evaluating the activity of a molecule for its capacity of being a substrate of PHEX comprising the steps of:

- contacting said molecule with the PHEX enzyme of claim 1 in substantially phosphate-free conditions; and
- observing a cleavage product of said molecule as an indication that the molecule is a PHEX substrate.

36. The method of claim 35, which further comprises the step of comparing said molecule with PTHrP107-139 as a positive control.

SUB A6

37. A method for evaluating PHEX activity in a sample which comprises the steps of contacting the sample with a substrate as defined in claim 35 or 36, or preferably with PTHrP107-139, in substantially phosphate-free conditions and observing the apparition of a cleavage product of said substrate or PTHrP107-139 as an indication of PHEX activity in the sample.

38. The method of claim 37, which further comprises a step of comparing said PHEX activity in the sample with the activity of the PHEX enzyme of claim 1 as a positive control.

SUB A7

39. A method for evaluating the activity of a molecule for its capacity of being an inhibitor of PHEX comprising the steps of:

- contacting said molecule with a substrate as defined in claim 35 or 36, or preferably with PTHrP107-139, and the PHEX enzyme of claim 1 in substantially phosphate-free conditions; and
- observing an inhibition of the formation of a cleavage product as an indication that said molecule is a PHEX inhibitor.

SUB A7 CONT.

40. A kit for executing the method of any one of claims 35 to 39.